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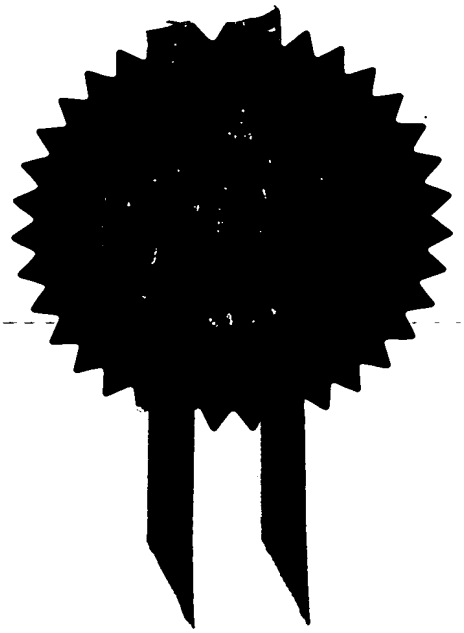

Signed A M Bennett.

Dated 22 August 2000

GB9919778.2

By virtue of a direction given under Section of the Patents Act 1977, the application is proceeding in the name of

ASTRAZENECA AB,
Incorporated in Sweden,
S-151 85 Sodertalje,
Sweden

[ADP No. 07822448003]

GB9919778.2

SECTION 15(9)(A)(I) (1977 ACT) APPLICATION FILED 4/9/2000

By virtue of a direction given under Section of the Patents Act 1977, the application is proceeding in the name of

ASTRAZENECA UK LIMITED
Incorporated in the United Kingdom
15 Stanhope Gate
LONDON
W1Y 6LN
United Kingdom

[ADP No. 07810294001]

Patent Form 1/77

.ents Act 1977
(Rule 16)

The Patent Office

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference — PHM 99-115

2. Patent application number *(The Patent Office will fill in this part)* — 21 AUG 1999 — **9919778.2**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Zeneca Limited
15 Stanhope Gate
LONDON W1Y 6LN
Great Britain

THE PATENT OFFICE
APPLICATION FILED
RECEIVED BY POST

Patents ADP number *(if you know it)*

6254007002

If the applicant is a corporate body, give the country/state of its incorporation

SECTION 1(1977 ACT)

4. Title of the invention — CHEMICAL COMPOUNDS

5. Name of your agent *(if you have one)*

TAIT, Brian Steele

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Global Intellectual Property, Patents
AstraZeneca PLC
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4TG
Great Britain

7726276001

Patents ADP number *(if you know it)*

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Patent Form 1/77

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Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Lynda M Slack Date 20 Aug 99

Zeneca Limited Authorised Signatory

12. Name and daytime telephone number of person to contact in the United Kingdom: Lynda M Slack 01625 516173

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CHEMICAL COMPOUNDS

The invention relates to pyrimidine derivatives, or pharmaceutically acceptable salts or *in vivo* hydrolysable esters thereof, which possess cell-cycle inhibitory activity and are accordingly useful for their anti-cell-proliferation (such as anti-cancer) activity and are therefore useful in methods of treatment of the human or animal body. The invention also relates to processes for the manufacture of said pyrimidine derivatives, to pharmaceutical compositions containing them and to their use in the manufacture of medicaments of use in the production of an anti-cell-proliferation effect in a warm-blooded animal such as man.

A family of intracellular proteins called cyclins play a central role in the cell cycle. The synthesis and degradation of cyclins is tightly controlled such that their level of expression fluctuates during the cell cycle. Cyclins bind to cyclin-dependent serine/threonine kinases (CDKs) and this association is essential for CDK (such as CDK1, CDK2, CDK4 and/or CDK6) activity within the cell. Although the precise details of how each of these factors combine to regulate CDK activity is poorly understood, the balance between the two dictates whether or not the cell will progress through the cell cycle.

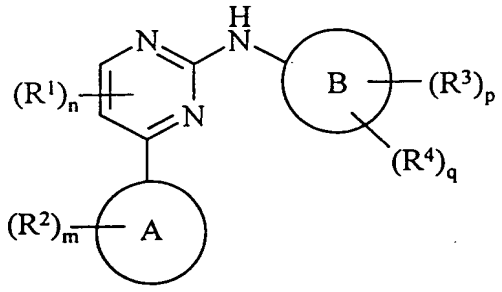
The recent convergence of oncogene and tumour suppressor gene research has identified regulation of entry into the cell cycle as a key control point of mitogenesis in tumours. Moreover, CDKs appear to be downstream of a number of oncogene signalling pathways. Disregulation of CDK activity by upregulation of cyclins and/or deletion of endogenous inhibitors appears to be an important axis between mitogenic signalling pathways and proliferation of tumour cells.

Accordingly it has been recognised that an inhibitor of cell cycle kinases, particularly inhibitors of CDK2, CDK4 and/or CDK6 (which operate at the S-phase, G1-S and G1-S phase respectively) should be of value as a selective inhibitor of cell proliferation, such as growth of mammalian cancer cells.

The present invention is based on the discovery that certain pyrimidine compounds surprisingly inhibit the effects of cell cycle kinases showing selectivity for CDK2, CDK4 and CDK6, and thus possess anti-cell-proliferation properties. Such properties are expected to be of value in the treatment of disease states associated with aberrant cell cycles and cell proliferation such as cancers (solid tumours and leukemias), fibroproliferative and

differentiative disorders , psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, atherosclerosis, arterial restenosis, autoimmune diseases, acute and chronic inflammation, bone diseases and ocular diseases with retinal vessel proliferation.

Accordingly, the present invention provides a compound of formula **(I)**:



(I)

wherein:

Ring A is imidazo[1,2a]pyrid-3-yl or pyrazolo[2,3a]pyrid-3-yl;

R^2 is attached to a ring carbon and is selected from halo, nitro, cyano, hydroxy, trifluoromethyl, trifluoromethoxy, amino, carboxy, carbamoyl, mercapto, sulphamoyl, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, *N*-(C_{1-6}alkyl)amino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkylS(O)$_a$ wherein a is 0 to 2, C_{1-6}alkoxycarbonyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl;

m is 0-5; wherein the values of R^2 may be the same or different;

R^1 is halo, nitro, cyano, hydroxy, trifluoromethyl, trifluoromethoxy, amino, carboxy, carbamoyl, mercapto, sulphamoyl, C_{1-3}alkyl, C_{2-3}alkenyl, C_{2-3}alkynyl, C_{1-3}alkoxy, C_{1-3}alkanoyl, *N*-(C_{1-3}alkyl)amino, *N,N*-(C_{1-2}alkyl)$_2$amino, C_{1-3}alkanoylamino, *N*-(C_{1-3}alkyl)carbamoyl, *N,N*-(C_{1-2}alkyl)$_2$carbamoyl, C_{1-3}alkylS(O)$_a$ wherein a is 0 to 2, *N*-(C_{1-3}alkyl)sulphamoyl or *N,N*-(C_{1-3}alkyl)$_2$sulphamoyl;

n is 0 to 2, wherein the values of R^1 may be the same or different;

Ring B is phenyl or phenyl fused to a C_{5-7}cycloalkyl ring;

R^3 is halo, nitro, cyano, hydroxy, amino, carboxy, carbamoyl, mercapto, sulphamoyl, C_{2-6}alkenyl or C_{2-6}alkynyl;

p is 0-4; wherein the values of R^3 may be the same or different;

R^4 is a group A-E-; wherein

A is optionally substituted on carbon by one or more D and is selected from C_{1-6}alkyl, phenyl, a heterocyclic group, phenylC_{1-6}alkyl or (heterocyclic group)C_{1-6}alkyl;

E is a direct bond or -O-, -C(O)-, -OC(O)-, -C(O)O-, -N(R^a)C(O)-, -C(O)N(R^a)-, -N(R^a)-, -S(O)$_r$-, -SO_2N(R^a)- or -N(R^a)SO_2-; wherein R^a is hydrogen or C_{1-6}alkyl optionally substituted by one or more D and r is 0-2;

D is independently selected from oxo, halo, nitro, cyano, hydroxy, trifluoromethyl, trifluoromethoxy, amino, carboxy, carbamoyl, mercapto, sulphamoyl, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, *N*-(C_{1-6}alkyl)amino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkylS(O)$_a$ wherein a is 0 to 2, C_{1-6}alkoxycarbonyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl; and

q is 0-2; wherein the values of R^4 maybe the same or different; and wherein $p + q \leq 5$; or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof.

In this specification the term "alkyl" includes both straight and branched chain alkyl groups but references to individual alkyl groups such as "propyl" are specific for the straight chain version only. For example, "C_{1-6}alkyl" includes C_{1-4}alkyl, C_{1-3}alkyl, propyl, isopropyl and *t*-butyl. However, references to individual alkyl groups such as 'propyl' are specific for the straight chained version only and references to individual branched chain alkyl groups such as 'isopropyl' are specific for the branched chain version only. A similar convention applies to other radicals, for example "phenylC_{1-6}alkyl" includes phenylC_{1-4}alkyl, benzyl, 1-phenylethyl and 2-phenylethyl. The term "halo" refers to fluoro, chloro, bromo and iodo.

Where optional substituents are chosen from "one or more" groups it is to be understood that this definition includes all substituents being chosen from one of the specified groups or the substituents being chosen from two or more of the specified groups.

A "heterocyclic group" is a saturated, partially saturated or unsaturated, mono or bicyclic ring containing 4-12 atoms of which at least one atom is chosen from nitrogen, sulphur or oxygen, which may, unless otherwise specified, be carbon or nitrogen linked, wherein a -CH_2- group can optionally be replaced by a -C(O)-, a ring nitrogen atom may optionally bear a C_{1-6}alkyl group and form a quaternary compound or a ring nitrogen and/or sulphur atom may be optionally oxidised to form the *N*-oxide and or the S-oxides. Examples

and suitable values of the term "heterocyclic group" are morpholino, piperidyl, pyridyl, pyranyl, pyrrolyl, isothiazolyl, indolyl, quinolyl, thienyl, 1,3-benzodioxolyl, thiadiazolyl, piperazinyl, thiazolidinyl, pyrrolidinyl, thiomorpholino, pyrrolinyl, homopiperazinyl, 3,5-dioxapiperidinyl, tetrahydropyranyl, imidazolyl, pyrimidyl, pyrazinyl, pyridazinyl, isoxazolyl, *N*-methylpyrrolyl, 4-pyridone, 1-isoquinolone, 2-pyrrolidone, 4-thiazolidone, pyridine-*N*-oxide and quinoline-*N*-oxide. Preferably a "heterocyclic group" is a saturated, partially saturated or unsaturated, mono or bicyclic ring containing 5 or 6 atoms of which at least one atom is chosen from nitrogen, sulphur or oxygen, which may, unless otherwise specified, be carbon or nitrogen linked, wherein a $-CH_2-$ group can optionally be replaced by a -C(O)-, a ring nitrogen atom may optionally bear a C_{1-6}alkyl group and form a quaternary compound or a ring nitrogen and/or sulphur atom may be optionally oxidised to form the *N*-oxide and or the S-oxides.

A suitable value for phenyl fused to a C_{5-7}cycloalkyl ring is indanyl or tetralinyl.

An example of "C_{1-6}alkanoyloxy" is acetoxy. Examples of "C_{1-6}alkoxycarbonyl" include methoxycarbonyl, ethoxycarbonyl, *n*- and *t*-butoxycarbonyl. Examples of "C_{1-6}alkoxy" include C_{1-3}alkoxy, methoxy, ethoxy and propoxy. Examples of "C_{1-6}alkanoylamino" include C_{1-3}alkanoylamino, formamido, acetamido and propionylamino. Examples of "C_{1-6}alkylS(O)$_a$ wherein a is 0 to 2" include C_{1-3}alkylS(O)$_a$, methylthio, ethylthio, methylsulphinyl, ethylsulphinyl, mesyl and ethylsulphonyl. Examples of "C_{1-6}alkanoyl" include C_{1-3}alkanoyl, propionyl and acetyl. Examples of "*N*-C_{1-6}alkylamino" include *N*-(C_{1-3}alkyl)amino, methylamino and ethylamino. Examples of "*N,N*-(C_{1-6}alkyl)$_2$amino" include *N,N*-(C_{1-2}alkyl)$_2$amino, di-*N*-methylamino, di-(*N*-ethyl)amino and *N*-ethyl-*N*-methylamino. Examples of "C_{2-6}alkenyl" are C_{2-3}alkenyl, vinyl, allyl and 1-propenyl. Examples of "C_{2-6}alkynyl" are C_{2-3}alkynyl, ethynyl, 1-propynyl and 2-propynyl. Examples of "*N*-(C_{1-6}alkyl)sulphamoyl" are *N*-(C_{1-3}alkyl)sulphamoyl, *N*-(methyl)sulphamoyl and *N*-(ethyl)sulphamoyl. Examples of "*N*-(C_{1-6}alkyl)$_2$sulphamoyl" are *N,N*-(C_{1-3}alkyl)$_2$sulphamoyl, *N,N*-(dimethyl)sulphamoyl and *N*-(methyl)-*N*-(ethyl)sulphamoyl. Examples of "*N*-(C_{1-6}alkyl)carbamoyl" are *N*-(C_{1-3}alkyl)carbamoyl, methylaminocarbonyl and ethylaminocarbonyl. Examples of "*N,N*-(C_{1-6}alkyl)$_2$carbamoyl" are *N,N*-(C_{1-2}alkyl)$_2$carbamoyl, dimethylaminocarbonyl and methylethylaminocarbonyl. Examples of "C_{5-7}cycloalkyl ring"

are cyclopropyl and cyclohexyl. Examples of "(heterocyclic group)C_{1-6}alkyl" include pyridylmethyl, 3-morpholinopropyl and 2-pyrimid-2-ylethyl.

A suitable pharmaceutically acceptable salt of a compound of the invention is, for example, an acid-addition salt of a compound of the invention which is sufficiently basic, for example, an acid-addition salt with, for example, an inorganic or organic acid, for example hydrochloric, hydrobromic, sulphuric, phosphoric, trifluoroacetic, citric or maleic acid. In addition a suitable pharmaceutically acceptable salt of a compound of the invention which is sufficiently acidic is an alkali metal salt, for example a sodium or potassium salt, an alkaline earth metal salt, for example a calcium or magnesium salt, an ammonium salt or a salt with an organic base which affords a physiologically-acceptable cation, for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

The compounds of the formula **(I)** may be administered in the form of a pro-drug which is broken down in the human or animal body to give a compound of the formula **(I)**. Examples of pro-drugs include *in vivo* hydrolysable esters of a compound of the formula **(I)**.

An *in vivo* hydrolysable ester of a compound of the formula **(I)** containing carboxy or hydroxy group is, for example, a pharmaceutically acceptable ester which is hydrolysed in the human or animal body to produce the parent acid or alcohol. Suitable pharmaceutically acceptable esters for carboxy include C_{1-6}alkoxymethyl esters for example methoxymethyl, C_{1-6}alkanoyloxymethyl esters for example pivaloyloxymethyl, phthalidyl esters, C_{3-8}cycloalkoxycarbonyloxyC_{1-6}alkyl esters for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolen-2-onylmethyl esters for example 5-methyl-1,3-dioxolen-2-onylmethyl; and C_{1-6}alkoxycarbonyloxyethyl esters for example 1-methoxycarbonyloxyethyl and may be formed at any carboxy group in the compounds of this invention.

An *in vivo* hydrolysable ester of a compound of the formula **(I)** containing a hydroxy group includes inorganic esters such as phosphate esters and α-acyloxyalkyl ethers and related compounds which as a result of the *in vivo* hydrolysis of the ester breakdown to give the parent hydroxy group. Examples of α-acyloxyalkyl ethers include acetoxymethoxy and 2,2-dimethylpropionyloxy-methoxy. A selection of *in vivo* hydrolysable ester forming groups for hydroxy include alkanoyl, benzoyl, phenylacetyl and substituted benzoyl and phenylacetyl, alkoxycarbonyl (to give alkyl carbonate esters), dialkylcarbamoyl and

N-(dialkylaminoethyl)-*N*-alkylcarbamoyl (to give carbamates), dialkylaminoacetyl a[redacted] carboxyacetyl. Examples of substituents on benzoyl include morpholino and piperazino linked from a ring nitrogen atom via a methylene group to the 3- or 4- position of the benzoyl ring.

Some compounds of the formula **(I)** may have chiral centres and/or geometric isomeric centres (E- and Z- isomers), and it is to be understood that the invention encompasses all such optical, diastereoisomers and geometric isomers that possess CDK inhibitory activity.

The invention relates to any and all tautomeric forms of the compounds of the formula **(I)** that possess CDK inhibitory activity.

It is also to be understood that certain compounds of the formula **(I)** can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which possess CDK inhibitory activity.

Preferred values of R^1, R^2, R^3, R^4, n, m, p, q, Ring A and Ring B are as follows. Such values may be used where appropriate with any of the definitions, claims or embodiments defined hereinbefore or hereinafter.

In one aspect of the invention preferably Ring A is imidazo[1,2a]pyrid-3-yl.

In another aspect of the invention preferably Ring A is pyrazolo[2,3a]pyrid-3-yl.

Preferably R^2 is attached to a ring carbon and is selected from halo, nitro, cyano, hydroxy, trifluoromethyl, trifluoromethoxy, amino, carboxy, carbamoyl, sulphamoyl, C_{1-3}alkyl, C_{2-3}alkenyl, C_{1-3}alkoxy, C_{1-3}alkanoyl, C_{1-3}alkanoyloxy, *N*-(C_{1-3}alkyl)amino, *N,N*-(C_{1-2}alkyl)$_2$amino, C_{1-3}alkanoylamino, *N*-(C_{1-3}alkyl)carbamoyl, *N,N*-(C_{1-2}alkyl)$_2$carbamoyl, C_{1-3}alkylS(O)$_a$ wherein a is 0 to 2, *N*-(C_{1-3}alkyl)sulphamoyl and *N,N*-(C_{1-3}alkyl)$_2$sulphamoyl.

More preferably R^2 is attached to a ring carbon and is C_{1-3}alkyl.

Particularly R^2 is attached to a ring carbon and is methyl.

Preferably m is 0-2; wherein the values of R^2 may be the same or different.

In one aspect of the invention preferably m is 0.

In another aspect of the invention preferably m is 1.

In a further aspect of the invention preferably m is 2; wherein the values of R^2 may be the same or different.

Preferably Ring A and $(R^2)_m$ together form imidazo[1,2a]pyrid-3-yl,

pyrazolo[2,3a]pyrid-3-yl, 2-methylimidazo[1,2a]pyrid-3-yl, 2-methylpyrazolo[2,3a]pyrid-3-yl or 2,5-dimethylimidazo[1,2a]pyrid-3-yl.

Preferably n is 0 or 1 and where n is 1 preferably R^1 is attached to the 5-position of the pyrimidine ring.

More preferably n is 0.

Preferably Ring B is phenyl or indanyl.

More preferably phenyl or indan-5-yl.

Particularly Ring B is phenyl.

Preferably R^3 is halo or sulphamoyl.

More preferably R^3 is fluoro, chloro, bromo or sulphamoyl.

Preferably p is 0-2; wherein the values of R^3 may be the same or different.

In one aspect of the invention preferably p is 0.

In another aspect of the invention preferably p is 1.

In a further aspect of the invention preferably p is 2; wherein the values of R^3 may be the same or different.

Preferably R^4 is a group A-E-; wherein

A is optionally substituted on carbon by one or more D and is selected from C_{1-4}alkyl, phenyl, a heterocyclic group or phenylC_{1-4}alkyl;

E is a direct bond or -O-, -C(O)-, -N(R^a)C(O)-, -S(O)$_r$- or -N(R^a)SO_2-; wherein R^a is hydrogen, methyl or ethyl and r is 0-2;

D is oxo, cyano, hydroxy, amino, carboxy, carbamoyl, sulphamoyl, C_{1-3}alkyl, C_{2-3}alkenyl, C_{2-3}alkynyl, C_{1-3}alkoxy, C_{1-3}alkanoyl, *N*-(C_{1-3}alkyl)amino, *N,N*-(C_{1-2}alkyl)$_2$amino, C_{1-3}alkanoylamino, *N*-(C_{1-3}alkyl)carbamoyl, *N,N*-(C_{1-2}alkyl)$_2$carbamoyl, C_{1-3}alkylS(O)$_a$ wherein a is 0 to 2, *N*-(C_{1-3}alkyl)sulphamoyl or *N,N*-(C_{1-3}alkyl)$_2$sulphamoyl.

More preferably R^4 is a group A-E-; wherein

A is optionally substituted on carbon by one or more D and is selected from C_{1-4}alkyl, phenyl, a heterocyclic group or phenylC_{1-4}alkyl;

E is a direct bond or -O-, -C(O)- or -S(O)$_r$-; wherein r is 0-2;

D is hydroxy or *N,N*-(C_{1-2}alkyl)$_2$amino.

Particularly R^4 is methyl, ethyl, methoxy, methylthio, mesyl, acetyl, 3-*N,N*-dimethylamino-2-hydroxypropoxy, 2-*N,N*-diethylaminoethoxy, benzyloxy, anilinosulphonyl, pyrimidin-2-ylaminosulphonyl, phenoxy, 3,5-dioxapiperidin-1-ylsulphonyl.

Preferably q is 0-1.

In one aspect of the invention q is 0.

In another aspect of the invention q is 1.

In a further aspect of the invention when Ring B is phenyl and q is 1, preferably R^4 is attached para to the -NH- moiety of formula **(I)**.

Preferably Ring B, $(R^3)_p$ $(R^4)_q$ and together form phenyl, 2-fluorophenyl, 3-fluorophenyl, 4-fluorophenyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 3-bromophenyl, 3-methylphenyl, 4-methylphenyl, 3-ethylphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 3-methylthiophenyl, 4-methylthiophenyl, 4-mesylphenyl, 3-sulphamoylphenyl, 4-sulphamoylphenyl, 3-acetylphenyl, 3,4-dichlorophenyl, 3-chloro-4-fluorophenyl, 2-chloro-4-methylphenyl, 4-(3-*N,N*-dimethylamino-2-hydroxypropoxy)phenyl, 4-benzyloxyphenyl, 4-anilinosulphonylphenyl, 4-(pyrimidin-2-ylsulphonyl)phenyl, 4-phenoxyphenyl, 4-(2-*N,N*-diethylaminoethoxy)phenyl, 4-(3,5-dioxapiperidin-1-ylsulphonyl)phenyl or indanyl.

Therefore in one aspect of the invention, there is provided a compound of formula **(I)** as depicted above wherein:

Ring A is imidazo[1,2a]pyrid-3-yl or pyrazolo[2,3a]pyrid-3-yl;

R^2 is attached to a ring carbon and is selected from halo, nitro, cyano, hydroxy, trifluoromethyl, trifluoromethoxy, amino, carboxy, carbamoyl, sulphamoyl, C_{1-3}alkyl, C_{2-3}alkenyl, C_{1-3}alkoxy, C_{1-3}alkanoyl, C_{1-3}alkanoyloxy, *N*-(C_{1-3}alkyl)amino, *N,N*-(C_{1-2}alkyl)$_2$amino, C_{1-3}alkanoylamino, *N*-(C_{1-3}alkyl)carbamoyl, *N,N*-(C_{1-2}alkyl)$_2$carbamoyl, C_{1-3}alkylS(O)$_a$ wherein a is 0 to 2, *N*-(C_{1-3}alkyl)sulphamoyl and *N,N*-(C_{1-3}alkyl)$_2$sulphamoyl;

m is 0-2; wherein the values of R^2 may be the same or different;

n is 0;

Ring B is phenyl or indanyl;

R^3 is halo or sulphamoyl;

R^4 is a group A-E-; wherein

A is optionally substituted on carbon by one or more D and is selected from C_{1-4}alkyl, phenyl, a heterocyclic group or phenylC_{1-4}alkyl;

E is a direct bond or -O-, -C(O)-, -N(R^a)C(O)-, -S(O)$_r$- or -N(R^a)SO_2-; wherein R^a is hydrogen, methyl or ethyl and r is 0-2;

p is 0-2; wherein the values of R^3 may be the same or different;

D is oxo, cyano, hydroxy, amino, carboxy, carbamoyl, sulphamoyl, C_{1-3}alkyl, C_{2-3}alkenyl, C_{2-3}alkynyl, C_{1-3}alkoxy, C_{1-3}alkanoyl, *N*-(C_{1-3}alkyl)amino, *N,N*-(C_{1-2}alkyl)$_2$amino, C_{1-3}alkanoylamino, *N*-(C_{1-3}alkyl)carbamoyl, *N,N*-(C_{1-2}alkyl)$_2$carbamoyl, C_{1-3}alkylS(O)$_a$ wherein a is 0 to 2, *N*-(C_{1-3}alkyl)sulphamoyl or *N,N*-(C_{1-3}alkyl)$_2$sulphamoyl;

q is 0-1; wherein the values of R^4 may be the same or different;

or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof.

Therefore in a further aspect of the invention, there is provided a compound of formula (I) as depicted above wherein:

Ring A is imidazo[1,2a]pyrid-3-yl or pyrazolo[2,3a]pyrid-3-yl;

R^2 is attached to a ring carbon and is C_{1-3}alkyl;

m is 0-2; wherein the values of R^2 may be the same or different;

n is 0;

Ring B is phenyl or indan-5-yl;

R^3 is fluoro, chloro, bromo or sulphamoyl;

p is 0-2; wherein the values of R^3 may be the same or different;

R^4 is methyl, ethyl, methoxy, methylthio, mesyl, acetyl, 3-*N,N*-dimethylamino-2-hydroxypropoxy, 2-*N,N*-diethylaminoethoxy, benzyloxy, anilinosulphonyl, pyrimidin-2-ylaminosulphonyl, phenoxy, 3,5-dioxapiperidin-1-ylsulphonyl.

q is 0-1; wherein the values of R^4 may be the same or different;

or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof.

Therefore in an additional aspect of the invention, there is provided a compound of formula (I) as depicted above wherein:

Ring A and $(R^2)_m$ together form imidazo[1,2a]pyrid-3-yl, pyrazolo[2,3a]pyrid-3-yl, 2-methylimidazo[1,2a]pyrid-3-yl, 2-methylpyrazolo[2,3a]pyrid-3-yl or 2,5-dimethylimidazo[1,2a]pyrid-3-yl;

n is 0;

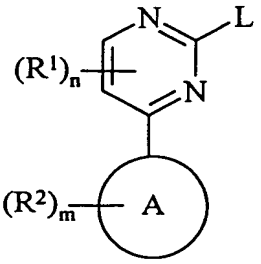
Ring B, $(R^3)_p$ and $(R^4)_q$ together form phenyl, 2-fluorophenyl, 3-fluorophenyl, 4-fluorophenyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 3-bromophenyl, 3-methylphenyl, 4-methylphenyl, 3-ethylphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 3-methylthiophenyl, 4-methylthiophenyl, 4-mesylphenyl, 3-sulphamoylphenyl, 4-sulphamoylphenyl, 3-acetylphenyl, 3,4-dichlorophenyl, 3-chloro-4-fluorophenyl, 2-chloro-4-methylphenyl, 4-(3-*N,N*-dimethylamino-2-hydroxypropoxy)phenyl, 4-benzyloxyphenyl, 4-anilinosulphonylphenyl, 4-(pyrimidin-2-ylsulphonyl)phenyl, 4-phenoxyphenyl, 4-(2-*N,N*-diethylaminoethoxy)phenyl, 4-(3,5-dioxapiperidin-1-ylsulphonyl)phenyl or indanyl; or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof.

In another aspect of the invention, preferred compounds of the invention are any one of Examples 1-38 or a pharmaceutically acceptable salt or an *in vivo* hydrolysable esters thereof.

Preferred aspects of the invention are those which relate to the compound of formula **(I)** or a pharmaceutically acceptable salt thereof.

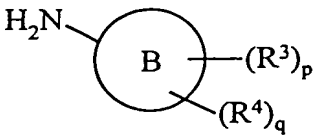
Another aspect of the present invention provides a process for preparing a compound of formula **(I)** or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof which process (wherein R^1, R^2, R^3, R^4, Ring A, Ring B, m, p, q and n are, unless otherwise specified, as defined in formula **(I)**) comprises of:

a) reaction of a pyrimidine of formula **(II)**:



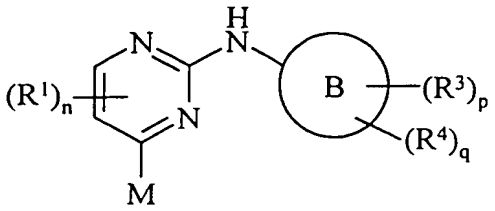
(II)

wherein L is a displaceable group; with an amine of formula **(III)**:



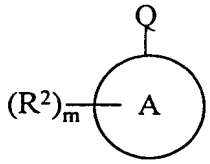
(III)

b) reacting a pyrimidine of formula **(IV)**:



(IV)

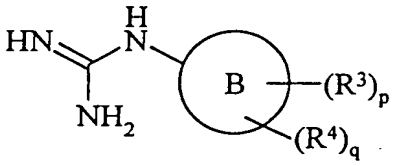
with a compound of the formula **(V)**:



(V)

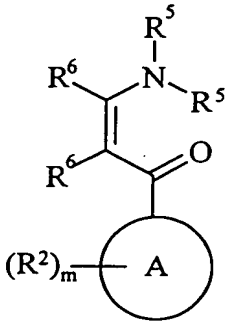
wherein one of M and Q is a displaceable group X and the other is an metallic reagent Y; or

c) reacting a compounds of formula **(VI)**:



(VI)

with a compound of formula **(VII)**:



(VII)

wherein R^5 is C_{1-6}alkyl and R^6 is hydrogen or R^1;

and thereafter if necessary:

i) converting a compound of the formula **(I)** into another compound of the formula **(I)**;

ii) removing any protecting groups;

iii) forming a pharmaceutically acceptable salt or *in vivo* hydrolysable ester.

L is a displaceable group, suitable values for L are for example, a halogeno or sulphonyloxy group, for example a chloro, bromo, methanesulphonyloxy or toluene-4-sulphonyloxy group.

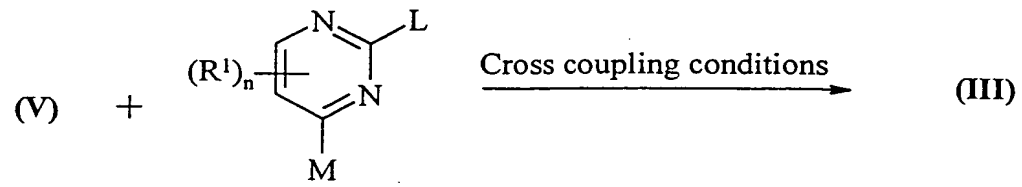
A suitable displaceable group X is, for example, a halogeno or sulphonyl group, for example a bromo, iodo or trifluoromethylsulphonyl group.

A suitable metallic group Y, is, for example, copper, lithium, an organoboron reagent such as $-B(OH)_2$, $-B(OPr^i)_2$ or $-B(Et)_2$, or an organotin compound such as $SnBu_3$, an organosilicon compound such as $Si(Me)F_2$, an organozirconium compound such as $ZrCl_3$, an organoaluminium compound such as $AlEt_2$, an organomagnesium compound such as MgBr, an organozinc compound such as ZnCl or an organomercury compound such as HgBr.

Specific reaction conditions for the above reactions are as follows.

a) Pyrimidines of formula **(II)** and amines of formula **(III)** may be reacted together:
i) in the presence of a suitable solvent for example a ketone such as acetone or an alcohol such as ethanol or butanol or an aromatic hydrocarbon such as toluene or N-methyl pyrrolidine, optionally in the presence of a suitable acid such as those defined above (or a suitable Lewis acid) and at a temperature in the range of 0°C to reflux, preferably reflux; or
ii) under standard Buchwald conditions (for example see *J. Am. Chem. Soc.*, **118**, 7215; *J. Am. Chem. Soc.*, **119**, 8451; *J. Org. Chem.*, **62**, 1568 and 6066) for example in the presence of palladium acetate, in a suitable solvent for example an aromatic solvent such as toluene, benzene or xylene, with a suitable base for example an inorganic base such as caesium carbonate or an organic base such as potassium-t-butoxide, in the presence of a suitable ligand such as 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl and at a temperature in the range of 25 to 80°C.

Pyrimidines of the formula **(II)** may be prepared according to the following scheme:

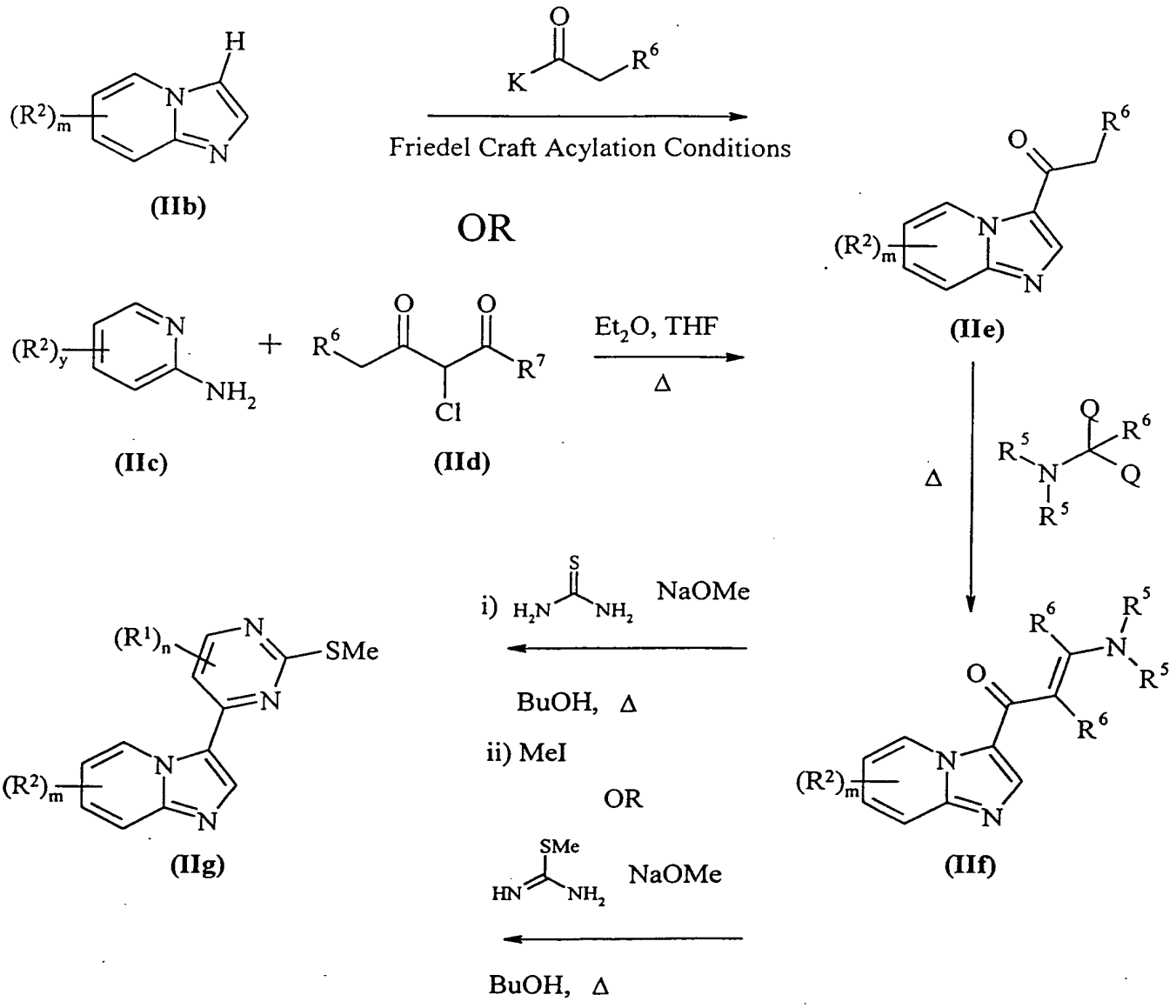


(IIa)

wherein one of M and Q is a displaceable group X as defined above and the other is an metallic reagent Y as defined above.

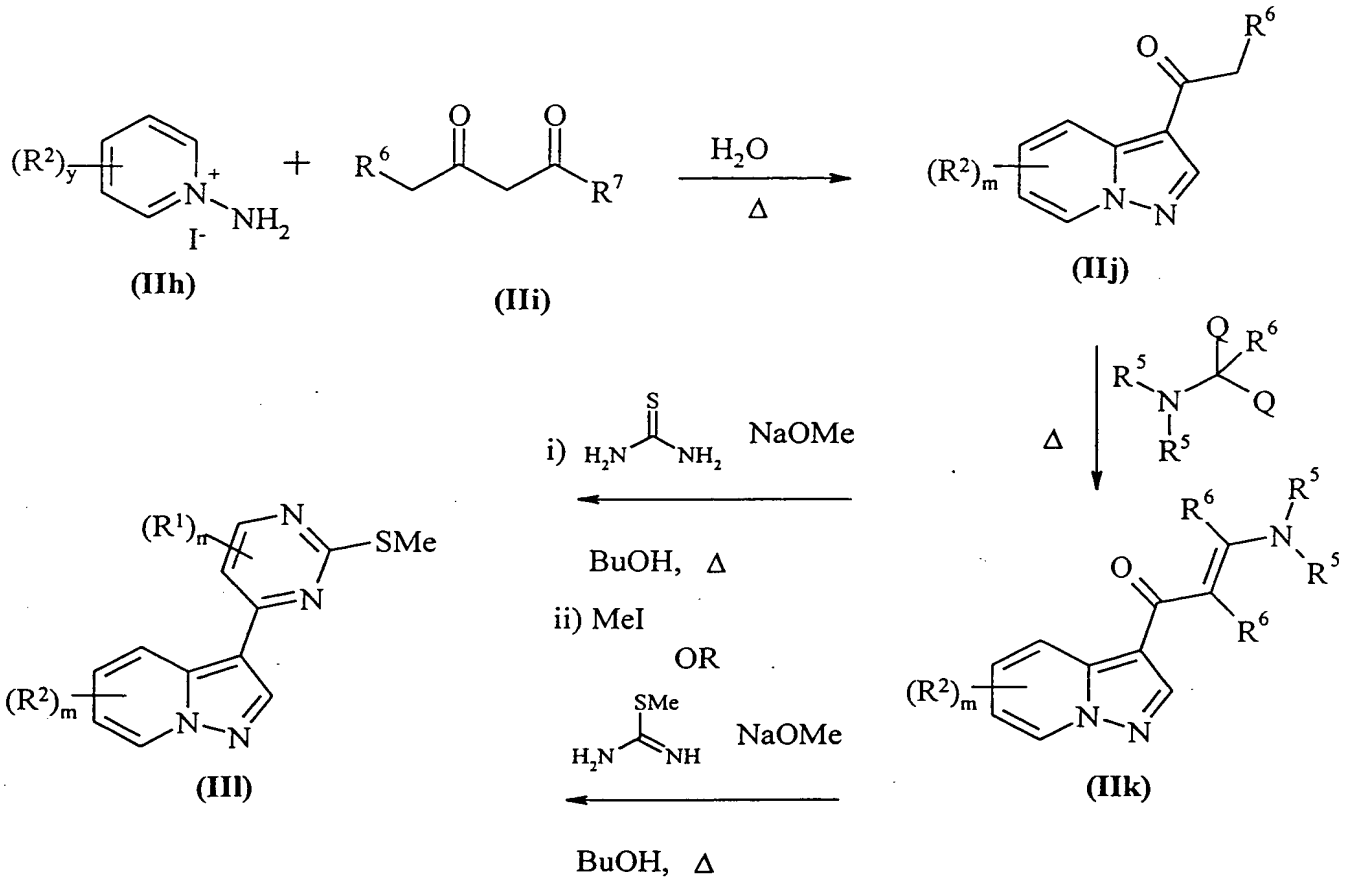
Cross coupling conditions are well known in the art. Suitable conditions include, for example, those described under b) below.

Where Ring A is imidazo[1,2a]pyrid-3-yl compounds of the formula **(II)** may also be prepared according to the following scheme:



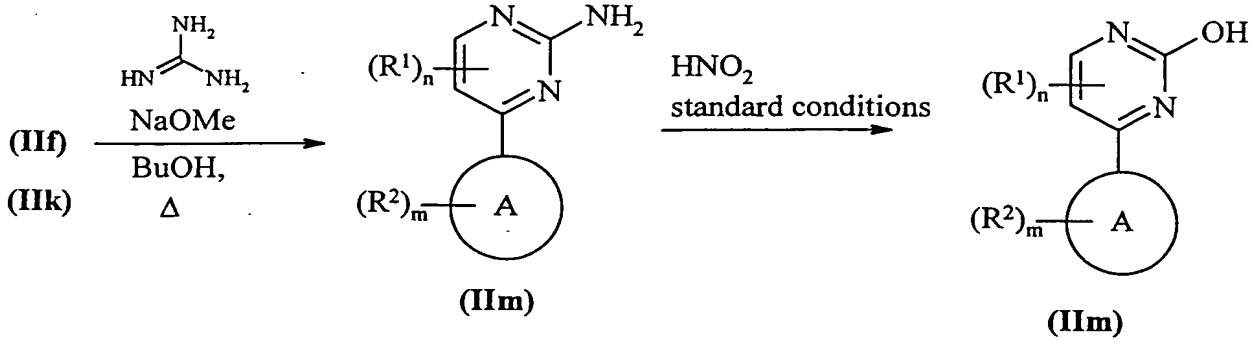
K is a suitable leaving group (for example C_{1-6}alkanoyloxy), R^6 is as defined above, y is 0-4, R^7 is hydrogen or R^2; Q is a suitable leaving group (for example C_{1-6}alkoxy) and R^5 is as defined above.

Where Ring A is pyrazolo[2,3a]pyrid-3-yl compounds of the formula **(II)** may also be prepared according to the following scheme:



wherein R^5, R^6 and R^7 are as defined above.

Compounds of formula **(IIf)** or **(IIk)** may be further modified to produce compounds of formula **(IIn)**:



It will be appreciated by those skilled in the art that compounds of formula **(IIn)** may be additionally modified by standard functional group modification reactions known in the art to produce compounds of formula **(II)** where L is other leaving groups for example chloro, bromo, tosyl and mesyl.

Compounds of formula **(IIa)**, **(IIb)**, **(IIc)**, **(IId)**, **(IIh)**, **(IIi)** and **(III)** are commercially available compounds, or they are known in the literature, or they are prepared by standard processes known in the art.

b) Compounds of formula (IV) and compounds of formula (V) may be reacted together under standard cross coupling conditions. Examples of these are in the presence of a catalyst, for example, a metallic catalyst such as tetrakis(triphenylphosphine)palladium(0), palladium(II) chloride, palladium(II) bromide, nickel(II) chloride, nickel(II) bromide or bis(triphenylphosphine)nickel(II) chloride, in the presence of a suitable inert solvent or diluent, for example tetrahydrofuran, 1,4-dioxan, 1,2-dimethoxyethane, benzene, toluene, xylene, methanol or ethanol. The reaction is preferably conducted in the presence of a suitable base such as, for example, sodium carbonate or potassium carbonate, pyridine, 4-dimethylaminopyridine, triethylamine or morpholine, and conveniently at a temperature in the range, for example 10 to 250°C, preferably in the range 60 to 120°C.

Compounds of formula **(IV)** may be prepared according to the following scheme:

$$\textbf{(IIa)} \quad + \quad \textbf{(III)} \quad \xrightarrow{\text{Conditions as process a)}} \quad \textbf{(IV)}$$

Compounds of formula (V) are commercially available compounds, or they are known in the literature, or they are prepared by standard processes known in the art.

c) compounds of formula **(VI)** and compounds of formula **(VII)** are reacted together in a suitable solvent such as *N*-methylpyrrolidinone or butanol at a temperature in the range of 100-200°C, preferably in the range of 150-170°C. The reaction is preferably conducted in the presence of a suitable base such as, for example, sodium methoxide or potassium carbonate.

Compounds of formula **(VI)** and **(VII)** are commercially available compounds, or they are known in the literature, or they are prepared by standard processes known in the art, or compounds of formula **(VII)** may be prepared by a process similar to that described for **(IIf)** and **(IIk)** hereinabove.

It will be appreciated that certain of the various ring substituents in the compounds of the present invention may be introduced by standard aromatic substitution reactions or generated by conventional functional group modifications either prior to or immediately following the processes mentioned above, and as such are included in the process aspect of the invention. Such reactions and modifications include, for example, introduction of a

substituent by means of an aromatic substitution reaction, reduction of substituents, alkylation of substituents and oxidation of substituents. The reagents and reaction conditions for such procedures are well known in the chemical art. Particular examples of aromatic substitution reactions include the introduction of a nitro group using concentrated nitric acid, the introduction of an acyl group using, for example, an acyl halide and Lewis acid (such as aluminium trichloride) under Friedel Crafts conditions; the introduction of an alkyl group using an alkyl halide and Lewis acid (such as aluminium trichloride) under Friedel Crafts conditions; and the introduction of a halogeno group. Particular examples of modifications include the reduction of a nitro group to an amino group by for example, catalytic hydrogenation with a nickel catalyst or treatment with iron in the presence of hydrochloric acid with heating; oxidation of alkylthio to alkylsulphinyl or alkylsulphonyl.

It will also be appreciated that in some of the reactions mentioned herein it may be necessary/desirable to protect any sensitive groups in the compounds. The instances where protection is necessary or desirable and suitable methods for protection are known to those skilled in the art. Conventional protecting groups may be used in accordance with standard practice (for illustration see T.W. Green, Protective Groups in Organic Synthesis, John Wiley and Sons, 1991). Thus, if reactants include groups such as amino, carboxy or hydroxy it may be desirable to protect the group in some of the reactions mentioned herein.

A suitable protecting group for an amino or alkylamino group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an alkoxycarbonyl group, for example a methoxycarbonyl, ethoxycarbonyl or *t*-butoxycarbonyl group, an arylmethoxycarbonyl group, for example benzyloxycarbonyl, or an aroyl group, for example benzoyl. The deprotection conditions for the above protecting groups necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or alkoxycarbonyl group or an aroyl group may be removed for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an acyl group such as a *t*-butoxycarbonyl group may be removed, for example, by treatment with a suitable acid as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid and an arylmethoxycarbonyl group such as a benzyloxycarbonyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon, or by treatment with a Lewis acid for example boron tris(trifluoroacetate). A suitable alternative protecting group

or a primary amino group is, for example, a phthaloyl group which may be removed by treatment with an alkylamine, for example dimethylaminopropylamine, or with hydrazine.

A suitable protecting group for a hydroxy group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an aroyl group, for example benzoyl, or an arylmethyl group, for example benzyl. The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or an aroyl group may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an arylmethyl group such as a benzyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a methyl or an ethyl group which may be removed, for example, by hydrolysis with a base such as sodium hydroxide, or for example a *t*-butyl group which may be removed, for example, by treatment with an acid, for example an organic acid such as trifluoroacetic acid, or for example a benzyl group which may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

The protecting groups may be removed at any convenient stage in the synthesis using conventional techniques well known in the chemical art.

As stated hereinbefore the compounds defined in the present invention possesses anti-cell-proliferation activity such as anti-cancer activity which is believed to arise from the CDK inhibitory activity of the compound. These properties may be assessed, for example, using the procedure set out below:-

Assay

The following abbreviations have been used :-

HEPES is *N*-[2-Hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]

DTT is Dithiothretiol

PMSF is Phenylmethylsulfonyl fluoride

The compounds were tested in an *in vitro* kinase assay in 96 well format using Scintillation Proximity Assay (SPA - obtained from Amersham) for measuring incorporation of [γ-33-P]-Adenosine Triphosphate into a test substrate (GST-Retinoblastoma protein; GST-Rb). In each well was placed the compound to be tested (diluted in DMSO and water to

correct concentrations) and in control wells either roscovitine as an inhibitor control or DM as a positive control.

Approximately 0.2µl of CDK2/Cyclin E partially-purified enzyme (amount dependent on enzyme activity) diluted in 25µl incubation buffer was added to each well then 20µl of GST-Rb/ATP/ATP33 mixture (containing 0.5µg GST-Rb and 0.2µM ATP and 0.14µCi [γ-33-P]-Adenosine Triphosphate in incubation buffer), and the resulting mixture shaken gently, then incubated at room temperature for 60 minutes.

To each well was then added 150µL stop solution containing (0.8mg/well of Protein A-PVT SPA bead (Amersham)), 20pM/well of Anti-Glutathione Transferase, Rabbit IgG (obtained from Molecular Probes), 61mM EDTA and 50mM HEPES pH 7.5 containing 0.05% sodium azide.

The plates were sealed with Topseal-S plate sealers, left for two hours then spun at 2500rpm, 1124xg., for 5 minutes. The plates were read on a Topcount for 30 seconds per well.

The incubation buffer used to dilute the enzyme and substrate mixes contained 50mM HEPES pH7.5, 10mM $MnCl_2$, 1mM DTT, 100µM Sodium vanadate, 100µM NaF, 10mM Sodium Glycerophosphate, BSA (1mg/ml final).

Test substrate

In this assay only part of the retinoblastoma protein (Science 1987 Mar13;235(4794):1394-1399; Lee W.H., Bookstein R., Hong F., Young L.J., Shew J.Y., Lee E.Y.) was used, fused to a GST tag. PCR of retinoblastoma gene encoding amino acids 379-928 (obtained from retinoblastoma plasmid ATCC pLRbRNL) was performed, and the sequence cloned into pGEX 2T fusion vector (Smith D.B. and Johnson, K.S. Gene 67, 31 (1988); which contained a tac promoter for inducible expression, internal lac I^q gene for use in any E.Coli host, and a coding region for thrombin cleavage - obtained from Pharmacia Biotech) which was used to amplify amino acids 792-928. This sequence was again cloned into pGEX 2T.

The retinoblastoma 792-928 sequence so obtained was expressed in E.Coli (BL21 (DE3) pLysS cells) using standard inducible expression techniques, and purified as follows.

E.coli paste was resuspended in 10ml/g of NETN buffer (50mM Tris pH 7.5, 120mM NaCl, 1mM EDTA, 0.5%v/v NP-40, 1mM PMSF, 1ug/ml leupeptin, 1ug/ml aprotinin and 1ug/ml pepstatin) and sonicated for 2 x 45 seconds per 100ml homogenate. After

centrifugation, the supernatant was loaded onto a 10ml glutathione Sepharose column (Pharmacia Biotech, Herts, UK), and washed with NETN buffer. After washing with kinase buffer (50mM HEPES pH 7.5, 10mM MgCl2, 1mM DTT, 1mM PMSF, 1ug/ml leupeptin, 1ug/ml aprotinin and 1ug/ml pepstatin) the protein was eluted with 50mM reduced glutathione in kinase buffer. Fractions containing GST-Rb(792-927) were pooled and dialysed overnight against kinase buffer. The final product was analysed by Sodium Dodeca Sulfate (SDS) PAGE (Polyacrylamide gel) using 8-16% Tris-Glycine gels (Novex, San Diego, USA).

CDK2 and Cyclin E

The open reading frames of CDK2 and Cyclin E were isolated by reverse transcriptase-PCR using HeLa cell and activated T cell mRNA as a template and cloned into the insect expression vector pVL1393 (obtained from Invitrogen 1995 catalogue number: V1392-20). CDK2 and cyclin E were then dually expressed [using a standard virus Baculogold co-infection technique] in the insect SF21 cell system (Spodoptera Frugiperda cells derived from ovarian tissue of the Fall Army Worm - commercially available).

Example production of Cyclin E/CDK2

The following Example provides details of the production of Cyclin E/CDK2 in SF21 cells (in TC100 + 10% FBS(TCS) + 0.2% Pluronic) having dual infection MOI 3 for each virus of Cyclin E & CDK2.

SF21 cells grown in a roller bottle culture to 2.33 x 10^6 cells/ml were used to inoculate 10 x 500 ml roller bottles at 0.2 x 10E6 cells/ml. The roller bottles were incubated on a roller rig at 28°C.

After 3 days (72 hrs.) the cells were counted, and the average from 2 bottles found to be 1.86 x 10E6 cells/ml. (99% viable). The cultures were then infected with the dual viruses at an MOI 3 for each virus.

The viruses were mixed together before addition to the cultures, and the cultures returned to the roller rig 28°C.

After 2 days (48 hrs.) post infection the 5 Litres of culture was harvested. The total cell count at harvest was 1.58 x 10E6 cells/ml.(99% viable). The cells were spun out at 2500rpm, 30 mins., 4°C in Heraeus Omnifuge 2.0 RS in 250 ml. lots. The supernatant was discarded.

Partial co-purification of Cdk2 and Cyclin E

Sf21 cells were resuspended in lysis buffer (50mM Tris pH 8.2, 10mM $MgCl_2$, 1mM DTT, 10mM glycerophosphate, 0.1mM sodium orthovanadate, 0.1mM NaF, 1mM PMSF, 1ug/ml leupeptin and 1ug/ml aprotinin) and homogenised for 2 minutes in a 10ml Dounce homgeniser. After centrifugation, the supernatant was loaded onto a Poros HQ/M 1.4/100 anion exchange column (PE Biosystems, Hertford, UK). Cdk2 and Cyclin E were coeluted at the beginning of a 0-1M NaCl gradient (run in lysis buffer minus protease inhibitors) over 20 column volumes. Co-elution was checked by western blot using both anti-Cdk2 and anti-Cyclin E antibodies (Santa Cruz Biotechnology, California, US).

By analogy, assays designed to assess inhibition of CDK4 and CDK6 may be constructed. CDK2 (EMBL Accession No. X62071) may be used together with Cyclin A or Cyclin E (see EMBL Accession No. M73812), and further details for such assays are contained in PCT International Publication No. WO99/21845, the relevant Biochemical & Biological Evaluation sections of which are hereby incorporated by reference.

Although the pharmacological properties of the compounds of the formula **(I)** vary with structural change, in general activity possessed by compounds of the formula **(I)** may be demonstrated at IC_{50} concentrations or doses in the range 250μM to 1nM.

When tested in the above in-vitro assay the CDK2 inhibitory activity of Example 11 was measured as IC_{50} = 0.19μM and that of Example 12 as IC_{50} = 0.17μM.

The *in vivo* activity of the compounds of the present invention may be assessed by standard techniques, for example by measuring inhibition of cell growth and assessing cytotoxicity.

Inhibition of cell growth may be measured by staining cells with Sulforhodamine B (SRB), a fluorescent dye that stains proteins and therefore gives an estimation of amount of protein (i.e. cells) in a well (see Boyd, M.R.(1989) Status of the NCI preclinical antitumour drug discovery screen. Prin. Prac Oncol 10:1-12). Thus, the following details are provided of measuring inhibition of cell growth :-

Cells were plated in appropriate medium in a volume of 100μl in 96 well plates; media was Dulbecco's Modified Eagle media for MCF-7, SK-UT-1B and SK-UT-1. The cells were allowed to attach overnight, then inhibitor compounds were added at various concentrations in

a maximum concentration of 1% DMSO (v/v). A control plate was assayed to give a value for cells before dosing. Cells were incubated at 37°C, (5% CO_2) for three days.

At the end of three days TCA was added to the plates to a final concentration of 16% (v/v). Plates were then incubated at 4°C for 1 hour, the supernatant removed and the plates washed in tap water. After drying, 100µl SRB dye (0.4% SRB in 1% acetic acid) was added for 30 minutes at 37°C. Excess SRB was removed and the plates washed in 1% acetic acid. The SRB bound to protein was solubilised in 10mM Tris pH7.5 and shaken for 30 minutes at room temperature. The ODs were read at 540nm, and the concentration of inhibitor causing 50% inhibition of growth was determined from a semi-log plot of inhibitor concentration versus absorbance. The concentration of compound that reduced the optical density to below that obtained when the cells were plated at the start of the experiment gave the value for toxicity.

Typical IC_{50} values for compounds of the invention when tested in the SRB assay are in the range 1mM to 1nM.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a pyrimidine derivative of the formula **(I)**, or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof, as defined hereinbefore in association with a pharmaceutically-acceptable diluent or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) as a sterile solution, suspension or emulsion, for topical administration as an ointment or cream or for rectal administration as a suppository.

In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compound of formula **(I)** will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000 mg per square meter body area of the animal, i.e. approximately 0.1-100 mg/kg, and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250 mg of active ingredient. Preferably a daily dose in the range of 1-50 mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route

of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

According to a further aspect of the present invention there is provided a compound of the formula (**I**), or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof, as defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

We have found that the compounds defined in the present invention, or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof, are effective cell cycle inhibitors (anti-cell proliferation agents), which property is believed to arise from their CDK inhibitory properties. Accordingly the compounds of the present invention are expected to be useful in the treatment of diseases or medical conditions mediated alone or in part by CDK enzymes, i.e. the compounds may be used to produce a CDK inhibitory effect in a warm-blooded animal in need of such treatment. Thus the compounds of the present invention provide a method for treating the proliferation of malignant cells characterised by inhibition of CDK enzymes, i.e. the compounds may be used to produce an anti-proliferative effect mediated alone or in part by the inhibition of CDKs. Such a compound of the invention is expected to possess a wide range of anti-cancer properties as CDKs have been implicated in many common human cancers such as leukaemia and breast, lung, colon, rectal, stomach, prostate, bladder, pancreas and ovarian cancer. Thus it is expected that a compound of the invention will possess anti-cancer activity against these cancers. It is in addition expected that a compound of the present invention will possess activity against a range of leukaemias, lymphoid malignancies and solid tumours such as carcinomas and sarcomas in tissues such as the liver, kidney, prostate and pancreas. In particular such compounds of the invention are expected to slow advantageously the growth of primary and recurrent solid tumours of, for example, the colon, breast, prostate, lungs and skin. More particularly such compounds of the invention, or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof, are expected to inhibit the growth of those primary and recurrent solid tumours which are associated with CDKs, especially those tumours which are significantly dependent on CDKs for their growth and spread, including for example, certain tumours of the colon, breast, prostate, lung, vulva and skin.

It is further expected that a compound of the present invention will possess activity

against other cell-proliferation diseases in a wide range of other disease states including leukaemias, fibroproliferative and differentiative disorders, psoriasis, rheumatoid arthritis, Kaposi's sarcoma, haemangioma, acute and chronic nephropathies, atheroma, atherosclerosis, arterial restenosis, autoimmune diseases, acute and chronic inflammation, bone diseases and ocular diseases with retinal vessel proliferation.

Thus according to this aspect of the invention there is provided a compound of the formula (I), or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof, as defined hereinbefore for use as a medicament; and the use of a compound of the formula (I), or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof, as defined hereinbefore in the manufacture of a medicament for use in the production of a cell cycle inhibitory (anti-cell-proliferation) effect in a warm-blooded animal such as man. Particularly, an inhibitory effect is produced by preventing entry into or progression through the S phase by inhibition of CDK2, CDK4 and/or CDK6, especially CDK2.

According to a further feature of this aspect of the invention there is provided a method for producing a cell cycle inhibitory (anti-cell-proliferation) effect in a warm-blooded animal, such as man, in need of such treatment which comprises administering to said animal an effective amount of a compound as defined immediately above. Particularly, an inhibitory effect is produced by preventing entry into or progression through the S phase by inhibition of CDK2, CDK4 and/or CDK6, especially CDK2.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular cell-proliferation disease will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. A unit dose in the range, for example, 1-100 mg/kg, preferably 1-50 mg/kg is envisaged.

The CDK inhibitory activity defined hereinbefore may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the cell cycle inhibitory treatment defined hereinbefore may be:

surgery, radiotherapy or chemotherapy. Such chemotherapy may cover three main categories of therapeutic agent:

(i) other cell cycle inhibitory agents that work by the same or different mechanisms from those defined hereinbefore;

(ii) cytostatic agents such as antioestrogens (for example tamoxifen,toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors); and

(iii) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincrisitine and taxoids like taxol, taxotere); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan). According to this aspect of the invention there is provided a pharmaceutical product comprising a compound of the formula (I) as defined hereinbefore and an additional anti-tumour substance as defined hereinbefore for the conjoint treatment of cancer.

In addition to their use in therapeutic medicine, the compounds of formula (I) and their pharmaceutically acceptable salts are also useful as pharmacological tools in the development and standardisation of in vitro and *in vivo* test systems for the evaluation of the

effects of inhibitors of cell cycle activity in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

In the above other pharmaceutical composition, process, method, use and medicament manufacture features, the alternative and preferred embodiments of the compounds of the invention described herein also apply.

Examples

The invention will now be illustrated by the following non-limiting examples in which, unless stated otherwise:

(i) temperatures are given in degrees Celsius (°C); operations were carried out at room or ambient temperature, that is, at a temperature in the range of 18-25°C;

(ii) organic solutions were dried over anhydrous magnesium sulphate; evaporation of solvent was carried out using a rotary evaporator under reduced pressure (600-4000 Pascals; 4.5-30mmHg) with a bath temperature of up to 60°C;

(iii) chromatography means flash chromatography on silica gel; thin layer chromatography (TLC) was carried out on silica gel plates; where a silica Bond Elut column is referred to, this means a column containing 10g or 20g of silica of 40 micron particle size, the silica being contained in a 60ml disposable syringe and supported by a porous disc, obtained from Varian, Harbor City, California, USA under the name "Mega Bond Elut SI", "Mega Bond Elut" is a trademark;

(iv) in general, the course of reactions was followed by TLC and reaction times are given for illustration only;

(v) final products had satisfactory proton nuclear magnetic resonance (NMR) spectra and/or mass spectral data;

(vi) yields are given for illustration only and are not necessarily those which can be obtained by diligent process development; preparations were repeated if more material was required;

(vii) when given, NMR data is in the form of delta values for major diagnostic protons, given in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, determined at 300 MHz using perdeuterio dimethyl sulphoxide (DMSO-d_6) as solvent unless otherwise indicated;

(viii) chemical symbols have their usual meanings; SI units and symbols are used;

(ix) solvent ratios are given in volume:volume (v/v) terms; and

(x) mass spectra were run with an electron energy of 70 electron volts in the chemical ionization (CI) mode using a direct exposure probe; where indicated ionization was effected by electron impact (EI), fast atom bombardment (FAB) or electrospray (ESP); values for m/z are given; generally, only ions which indicate the parent mass are reported;

(xi) unless stated otherwise compounds containing an asymmetrically substituted carbon and/or sulphur atom have not been resolved;

(xii) where a synthesis is described as being analogous to that described in a previous example the amounts used are the millimolar ratio equivalents to those used in the previous example;

(xvi) the following abbreviations have been used:

NMP	1-methyl-2-pyrrolidinone;
DMF	*N,N*-dimethylformamide;
DMSO	dimethylsulphoxide;
THF	tetrahydrofuran; and
EA	elemental analysis.

Example 1

2-(3-Chloroanilino)-4-(2-methylimidazo[1,2a]pyrid-3-yl)pyrimidine

Sodium hydride (236mg of a 60% suspension in mineral oil, 5.9mmol) was added to a solution of 3-chloroaniline (496ml, 4.7mmol) in NMP (10ml) under nitrogen. The mixture was stirred for 30 minutes at ambient temperature and a solution of 4-(2-methylimidazo[1,2a] pyrid-3-yl)-2-methylthiopyrimidine (Method 1) (600mg, 2.3mmol) in NMP (2ml) was added. The mixture was heated at 150°C for 3 hours. The reaction mixture was allowed to cool diluted with water and extracted with ethyl acetate. The combined extracts were dried and the volatiles removed by evaporation. The residue was purified by chromatography on silica eluting with ethyl acetate/hexane (1:1) increasing in polarity to ethyl acetate/methanol (97:3). The purified product was triturated with ether and hexane, collected by filtration and dried to give the title compound (159mg, 21%). NMR: 2.62 (s, 3H), 6.98-7.04 (m, 2H), 7.12 (d, 1H), 7.25 (dd, 1H), 7.42 (dd, 1H), 7.59-7.64 (m, 2H), 8.02 (s, 1H), 8.55 (d, 1H), 9.72 (d, 1H), 9.84 (s, 1H).

Examples 2-12

Following the procedure of Example 1 and using the appropriate starting materials the following compounds were prepared.

Ex	Compound	NMR	m/z [MH]$^+$
2	2-(4-Sulphamoylanilino)-4-(2-methylimidazo[1,2a]pyrid-3-yl)pyrimidine	2.64 (s, 3H), 7.05 (dd, 1H), 7.15-7.20 (m, 3H), 7.44 (dd, 1H), 7.64 (d, 1H), 7.74 (d, 2H), 7.92 (d, 2H), 8.68 (d, 1H), 9.75 (d, 1H)	381
3 [1]	2-Anilino-4-(2-methylimidazo [1,2a]pyrid-3-yl)pyrimidine	2.64 (s, 3H), 6.92-7.00 (m, 2H), 7.08 (d, 1H), 7.30 (dd, 1H), 7.40 (dd, 1H), 7.60 (d, 1H), 7.72 (d, 2H), 8.50 (d, 1H), 9.60 (s, 1H), 9.75 (d, 1H)	302
4	2-(4-Chloroanilino)-4-(2-methylimidazo[1,2a]pyrid-3-yl)pyrimidine	2.75 (s, 3H), 6.82 (dd, 1H), 7.01 (d, 1H), 7.22 (br s, 1H), 7.30 (m, 3H), 7.60 (m, 2H), 8.47 (d, 1H), 9.53 (d, 1H)	336
5 [1]	2-(3-Chloroanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.02 (d, 1H), 7.12 (dd, 1H), 7.30 (dd, 1H), 7.42 (d, 1H), 7.50 (dd, 1H), 7.60 (d, 1H), 7.75 (d, 1H), 8.00 (s, 1H), 8.48 (d, 1H), 8.61 (s, 1H), 9.82 (s, 1H)	322
6 [1]	2-(3,4-Dichloroanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.15 (dd, 1H), 7.50 (dd, 2H), 7.58 (d, 1H), 7.65 (dd, 1H), 7.78 (d, 1H), 8.22 (d, 1H), 8.50 (d, 1H), 8.62 (s, 1H), 9.95 (s, 1H)	
7	2-(4-Sulphamoylanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.20 (d, 3H), 7.55 (d, 2H), 8.80 (d, 3H), 8.95 (d, 2H), 8.50 (d, 1H), 8.68 (s, 1H), 10.05 (s, 1H), 10.10 (d, 1H)	367
8 [1]	2-(3-Chloro-4-fluoroanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.14 (dd, 1H), 7.32-7.55 (m, 3H), 7.60 (dd, 1H), 7.78 (d, 1H), 8.10 (dd, 1H), 8.48 (d, 1H), 8.62 (s, 1H), 9.82 (s, 1H)	340

9	2-(2-Chloroanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.08 (dd, 1H), 7.17 (d, 1H), 7.37 (m, 2H), 7.48 (dd, 1H), 7.51 (br s, 1H), 7.62 (d, 1H), 7.76 (d, 1H), 8.30 (s, 1H), 8.40 (m, 1H), 9.81 (d, 1H), 9.94 (dd, 1H)	322
10	2-(2-Chloro-4-methylanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	2.38 (s, 3H), 6.91 (dd, 1H), 7.14 (d, 1H), 7.28 (br s, 1H), 7.38 (m, 2H), 7.61 (s, 1H), 7.73 (d, 1H), 8.16 (d, 1H), 8.28 (s, 1H), 8.40 (d, 1H), 9.78 (d, 1H)	336
11 [1]	2-[4-(3,5-Dioxapiperidin-1-yl)sulphonylanilino]-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	4.87 (s, 2H), 5.20 (s, 4H), 7.16 (dd, 1H), 7.51 (d, 2H), 7.75 (d, 1H), 7.83 (d, 2H), 7.98 (d, 2H), 8.50 (d, 1H), 8.64 (s, 1H)	439
12 [1,2]	2-(4-(2-Diethylaminoethoxy) anilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	0.98 (t, 6H), 2.50-2.62 (m, 4H), 2.78-2.82 (m, 2H), 4.00 (t, 2H), 6.84 (dd, 2H), 7.08 (dd, 1H), 7.38 (d, 1H), 7.48 (dd, 1H), 7.60 (s, 2H), 7.75 (d, 1H), 8.38 (d, 1H), 8.59 (s, 1H), 9.42 (s, 1H)	403

[1] Sodium bis(trimethylsilyl)amide (1M solution in THF) was used in place of sodium hydride.

[2] The product was purified by chromatography on silica, eluting with dichloromethane / methanol (100:0 increasing to 80:20), triturated with ether and hexane and collected by filtration.

Example 13

2-[4-(3-Dimethylamino-2-hydroxypropoxy)anilino]-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine

A mixture of 4-(3-dimethylamino-2-hydroxypropoxy)aniline (497mg, 1.76mmol) (Method 11) and cyanamide (185mg, 4.4mmol) in NMP (1ml) were heated at 160°C for 30 minutes. A mixture of 3-(3-dimethylaminoprop-2-en-1-oyl)imidazo[1,2a]pyridine (Method 5) (400mg, 1.76mmol) and sodium methoxide (183mg, 3.5mmol) in 1-butanol (10ml) was then added and the mixture heated at reflux for 3 hours. The mixture was allowed to cool and the residue was purified by chromatography on silica, eluting with ethyl acetate/methanol (97:3 increasing in polarity to 90:10) to give the title compound (30mg, 4%). NMR: 2.35 (s, 6H),

.40-2.63 (m, 2H), 3.82-4.02 (m, 3H), 6.90 (d, 2H), 7.06 (dd, 1H), 7.30 (d, 1H), 7.50 (dd, 1H), 7.59 (s, 2H), 7.74 (d, 1H), 8.38 (d, 1H), 8.58 (s, 1H), 9.42 (s, 1H); m/z: 405 $[MH]^+$.

Examples 14-15

Following the procedure of Example 13 and using the appropriate starting materials the following compounds were prepared.

Ex	Compound	NMR	m/z $[MH]^+$
14 [1]	2-(4-(3-Dimethylamino-2-hydroxypropoxy)anilino)-4-(2-methylpyrazolo[2,3a]pyrid-3-yl)pyrimidine	2.20 (s, 6H), 2.26-2.45 (m, 2H), 2.65 (s, 3H), 3.80-3.95 (m, 3H), 4.80 (s, 1H), 6.88 (d, 2H), 7.00 (d, 2H), 7.38 (dd, 1H), 7.60 (d, 2H), 8.38 (d, 1H), 8.44 (d, 1H), 8.65 (d, 1H), 9.21 (s, 1H)	419
15 [2]	2-(4-(3-Dimethylamino-2-hydroxypropoxy)anilino)-4-(2-methylimidazo[1,2,a]pyrid-3-yl)pyrimidine	2.63 (s, 3H), 2.80 (s, 6H), 3.12-3.26 (m, 2H), 4.27 (br s, 1H), 5.93 (br s, 1H), 6.90-7.04 (m, 4H), 7.40 (t, 1H), 7.60 (dd, 2H), 8.45 (d, 1H), 9045 (s, 1H), 9.73 (d, 1H)	419

[1] Product was purified by chromatography on silica eluting with dichloromethane/hexane (1:1) increasing in polarity to dichloromethane/methanol/triethylamine (96:4:0.5).

2 Product was purified by chromatography on silica eluting with dichloromethane/methanol/ triethylamine (96:4:0.5) and recrystallized from acetonitrile/methanol.

Examples 16-36

The following examples were prepared, purified and characterised by the following generic method:

Sodium bis (trimethylsilyl)amide (2.05ml of a 1M solution in THF, 2.05mmol) was added to a solution of the aniline (1.65mmol) in NMP (1.5ml) under nitrogen. The mixture was stirred for 30 minutes at ambient temperature and a solution of 4-(imidazo[1,2a]pyrid-3-yl)-2-methylthiopyrimidine (Method 4) (200mg, 0.83mmol) in NMP (1ml) was added. The reaction mixture was heated at 150°C for 2.5 hours. The solvent and volatiles were removed

by evaporation and the residue was purified by chromatography on silica eluting with ethyl acetate, then ethyl acetate/methanol (97:3) and finally ethyl acetate/methanol (97:3). The reaction products were characterised by HPLC on a 4.6mm x 10cm Hichrom RPB 100A column eluting water/acetonitrile/formic acid (95:5:0.1 for 1.5 minutes then on a 10 minute gradient to 5:95:0.1) with a flow rate of 1.0ml/minute, detecting at 254nm (bandwidth 10nm).

Ex	Compound	HPLC Ret Time (mins)	M/z $[MH]^+$
16	2-Anilino-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.26	288
17	2-(2-Fluoroanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.26	306
18	2-(3-Bromoanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	8.30	368
19	2-(3-Fluoroanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.70	306
20	2-(3-Methoxyanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.39	318
21	2-(3-Methylthioanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.98	334
22	2-(3-Acetylanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.13	330
23	2-(3-Ethylanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	8.11	316
24	2-(4-Fluoroanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.47	306
25	2-(4-Chloroanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	8.15	322
26	2-(4-Methoxyanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.02	318
27	2-(4-Benzyloxyanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	8.65	394
28	2-(4-(Anilinosulphonyl)anilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.79	443
29	2-(4-(Mesyl)anilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	6.84	366
30	2-(4-Methylthioanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.89	334
31	2-(4-Methylanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.65	302
32	2-(3-Sulphamoylanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	6.30	367
33	2-[4-(Pyrimid-2-ylaminosulphonyl)anilino]-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	6.72	445
34	2-(4-Phenoxyanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	8.86	380

5	2-(3-Methylanilino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	7.63	302
36	2-(5-Indanylamino)-4-(imidazo[1,2a]pyrid-3-yl)pyrimidine	8.20	328

Example 37

2-(3-Chloroanilino)-4-(2,5-dimethylimidazo[1,2a]pyrid-3-yl)pyrimidine

2-Methylthio-4-(2,5-dimethylimidazo[1,2a]pyrid-3-yl)pyrimidine (Method 14) (200mg, 0.74 mmol) was added to a solution of 3-chloroaniline (0.16ml, 1.48mmol) and sodium hydride (60mg, 1.48mmol) in NMP (1ml) under nitrogen. The mixture was heated at 150°C for 4 hours and then allowed to cool. The crude reaction mixture was loaded onto a Bond Elut column eluting with dichloromethane to remove the NMP and then with dichloromethane/methanol/methylamine (75:20:5) to elute the product. The product was further purified by chromatography on silica eluting with ethyl acetate/hexane (8:2) and then ethyl acetate to give the title compound (22mg, 9%). NMR: 2.27 (s, 3H), 2.61 (s, 3H), 7.01 (d, 1H), 7.12 (d, 1H), 7.30 (m, 2H), 7.56 (d, 1H), 7.62 (d, 1H), 8.57 (d, 1H), 9.41 (s 1H), 9.83 (s, 1H); m/z: 350 $[MH]^+$.

Example 38

Following the procedure of Example 37 and using the appropriate starting materials the following compound was prepared.

Ex	Compound	NMR	m/z $[MH]^+$
38	2-(3-Chloroanilino)-4-(2-methylpyrazolo[2,3a]pyrid-3-yl)pyrimidine	2.64 (s, 3H), 6.95-7.03 (m, 2H), 7.17 (d, 1H), 7.32 (d, 1H), 7.44 (dd, 1H), 7.58-7.64 (m, 2H), 8.04 (s, 1H), 8.57 (d, 1H), 9.72 (d, 1H), 9.84 (s, 1H)	336

Preparation of Starting Materials

The starting materials for the Examples above are either commercially available or are readily prepared by standard methods from known materials. For example the following reactions are illustrations but not limitations of the preparation of some of the starting materials used in the above reactions.

Method 1

4-(2-Methylimidazo[1,2a]pyrid-3-yl)-2-methylthiopyrimidine

A mixture of 3-(3-dimethylaminoprop-2-en-1-oyl)-2-methyl-imidazo[1,2a]pyridine (Method 2) (20g, 87mmol), thiourea (6.52g, 86mmol) and sodium methoxide (1.19g, 22mmol) in butanol (220ml) was heated at 85°C for two hours under nitrogen. Methyl iodide (2ml, 32mmol) was added and the mixture heated at 85°C for a further 1 hour. Methanol was added and the volatiles were removed by evaporation. The residue was purified by chromatography on silica eluting with ethyl acetate/methanol (100:0 increasing in polarity to 97:3) to give the title compound (16g, 71%). NMR: 2.59 (s, 1H), 2.62 (s, 3H), 7.10 (dd, 1H), 7.40 (dd, 1H), 7.42 (d, 1H), 7.63 (d, 1H), 8.62 (s, 1H), 9.54 (d, 1H), m/z: 257 $[MH]^+$.

Method 2

3-(3-Dimethylaminoprop-2-en-1-oyl)-2-methyl-imidazo[1,2a]pyridine

A mixture of 3-acetyl-2-methyl-imidazo[1,2a]pyridine (Method 3) (40g, 0.23mol) and *N,N*-dimethylformamide dimethyl acetal (200ml) was heated at reflux under nitrogen for 4 days. The volatiles were removed by evaporation, the residue was triturated with hot ether and the solid product collected by filtration to give the title compound (21g, 40%). NMR: 2.64 (s, 3H), 3.29 (s, 6H), 5.50 (d, 1H), 7.00 (dd, 1H), 7.38 (dd, 1H), 7.54 (d, 1H), 7.70 (d, 1H), 9.55 (d, 1H), m/z: 230 $[MH]^+$.

Method 3

3-Acetyl-2-methyl-imidazo[1,2a]pyridine

A mixture of 2-aminopyridine (60g,0.64mol) and 3-chloro-2,4-pentanedione (101.4g, 0.75mol) in ether (450ml) and THF (750ml) were heated at reflux for 12 hours, then left to stand at ambient temperature for 18 hours. The solvent was removed by evaporation and the residue was purified by chromatography on silica eluting with dichloromethane/hexane (1:1) increasing in polarity to dichloromethane /methanol (98:2). The purified product was triturated with hexane to give the title compound (46.2g, 40%). NMR: 2.55 (s, 3H), 2.68 (s, 3H), 7.15 (dd, 1H), 7.56 (dd, 1H), 7.64 (d, 1H), 9.58 (d, 1H), m/z: 175 $[MH]^+$.

Method 4

4-(Imidazo[1,2a]pyrid-3-yl)-2-methylthiopyrimidine

A mixture of 3-(3-dimethylaminoprop-2-en-1-oyl)imidazo[1,2a]pyridine (Method 5) (0.90g, 4.2mmol), thiourea (0.32g, 4.2mmol) and sodium methoxide (0.34g, 6.3mmol) was heated at 85°C in n-butanol (10ml) for 2 hours. The mixture was allowed to cool to 30°C, methyl iodide (0.6ml, 9.6mmol) was added dropwise and stirring continued for a further 3 hours. The volatiles were removed by evaporation and the residue purified by chromatography on silica, eluting with ethyl acetate/methanol (100:0 increasing in polarity to 97:3) to give the title compound (0.94g, 93 %). NMR: 2.61 (s, 3H), 7.22 (dd, 1H), 7.54 (dd, 1H), 7.72 (d, 1H), 7.77 (d, 1H), 8.56 (d, 1H), 8.66 (s, 1H), 9.83 (d, 1H); m/z: 243 $[MH]^+$.

Method 5

3-(3-Dimethylaminoprop-2-en-1-oyl)imidazo[1,2a]pyridine

A mixture of crude 3-acetyl-3-imidazo[1,2,a]pyridine (Method 6) (3.3g, 19.1mmol) and *N,N*-dimethylformamide dimethyl acetal (40ml) was heated at reflux for 60 hours. The mixture was allowed to cool, the volatiles were removed by evaporation and the residue triturated with hot ether. The solid product was collected by filtration to give the title compound 2.29g, 52%. NMR: 2.90 (br s, 3H), 3.10 (br s, 3H), 5.81 (d, 1H), 7.09 (dd, 1H), 7.42 (dd, 1H), 7.65 (d, 1H), 7.70 (d, 1H), 8.43 (s, 1H), 9.72 (d, 1H); m/z: 216 $[MH]^+$.

Method 6

3-Acetylimidazo[1,2a]pyridine

Aluminium chloride (20.4g, 153.2mmol) was added in small portions to a solution of imidazo[1,2a]pyridine (8.9g, 75.7mmol) in dichloromethane (150ml) cooled at 5°C. The mixture was then allowed to warm to ambient temperature and stirred for 1 hour and then heated to reflux. Acetic anhydride (5.1ml, 53.9mmol) was then added slowly over 30 minutes and the mixture heated at reflux for further 90 minutes. The mixture was allowed to cool, the solvent was removed by evaporation and ice/water added to the residue. The aqueous mixture was made alkaline with 2M aqueous sodium hydroxide solution and extracted with ethyl acetate. The combined extracts were dried and the volatiles removed by evaporation to give a brown oil. This oil was shown to consist of ~35% of the title compound, the remainder being

imidazo[1,2,a]pyridine. This mixture was used without further purification. NMR: 2.57 (3H), 7.22 (dd, 1H), 7.61 (dd, 1H), 7.79 (d, 1H), 8.60 (s, 1H), 9.52 (d, 1H).

Method 7

4-(3,5-Dioxapiperidin-1-yl)sulphonylaniline

A mixture of 1-(3,5-dioxapiperidin-1-yl)sulphonyl-4-nitrobenzene (Method 8) (500mg, 1.82mmol) and 10% palladium on charcoal catalyst (150mg) in ethanol (25ml) and ethyl acetate (25ml) was stirred under an atmosphere of hydrogen for 3 hours. The catalyst was removed by filtration through diatomaceous earth and the filter pad was washed with ethanol and ethyl acetate. The volatiles were removed from the filtrate by evaporation and the residue triturated with ether and hexane to give the title compound (395mg, 88%). NMR: 4.90 (s, 2H), 5.10 (s, 4H), 6.02 (s, 2H), 6.58 (d, 2H), 7.50 (d, 2H).

Method 8

1-(3,5-Dioxapiperidin-1-yl)sulphonyl-4-nitrobenzene

4-Nitrobenzenesulphonamide (2.02g, 10mmol) was added to a solution of 1,3,5-trioxane (1.96g, 20mmol) in acetic acid (5ml). The mixture was stirred for 5 minutes and methanesulphonic acid (10ml) was added slowly. The mixture was then stirred at 35°C for 20 minutes, cooled to 0°C, diluted with water and extracted with ethyl acetate. The combined extracts were washed twice with water and twice with 5% aqueous sodium hydrogen carbonate solution, then dried and the volatiles removed by evaporation. The residue was recrystallized from ethanol to give the title compound (955mg, 35%). NMR: 4.87 (s, 2H), 5.30 (s, 4H), 8.20 (d, 2H), 8.42 (d, 2H).

Method 9

4-(2-Diethylaminoethoxy)aniline

A mixture of 4-(2-diethylaminoethoxy)-1-nitrobenzene (Method 10) (1.0g, 4.2mmol) and 10% palladium on charcoal catalyst (200mg) in ethanol (30ml) was stirred under an atmosphere of hydrogen for 3 hours. The catalyst was removed by filtration through diatomaceous earth and the filter pad was washed with methanol. The volatiles were removed

rom the filtrate by evaporation to give the title compound (400mg, 46%) as an oil. M/z: 209 $[MH]^+$.

Method 10

4-(2-Diethylaminoethoxy)-1-nitrobenzene

Water (8ml) and xylene (35ml) were added to a mixture of sodium 4-nitrophenoxide (10.5g, 65mmol), 2-(diethylamino)ethylchloride hydrochloride (8.6g, 50mmol) and potassium carbonate (10.4g, 75mmol) and the resulting mixture was heated at reflux for 2 hours. A Dean-Stark apparatus was then fitted and the water was removed. The organic solution was allowed to cool to ambient temperature and left to stand for 18 hours. The solution was decanted from the precipitated solid and the volatiles were removed from the decanted solution by evaporation to give the title compound (8.0g, 52%) as an oil. NMR: 0.90 (t, 6H), 2.50 (q, 2H), 2.89 (t, 2H), 4.15 (t, 2H), 7.15 (d, 2H), 8.18 (d, 2H); m/z: 239 $[MH]^+$.

Method 11

4-[3-(*N*,*N*-Dimethyl)amino-2-hydroxypropoxy]aniline

3-*N*,*N*-Dimethylamino-2-hydroxy-3-(4-nitrophenoxy)propane (Method 12) (3.75 g) was dissolved in ethanol (40 ml). Under an atmosphere of nitrogen, 10% palladium-on-carbon (0.4g) was added. The nitrogen atmosphere was replaced by one of hydrogen and the reaction mixture was stirred overnight. The catalyst was removed by filtration through diatomaceous earth and the filtrate was evaporated to dryness. The residue was dissolved in diethyl ether containing a small amount of isopropanol and hydrogen chloride solution (1M in ether, 16 ml) was added. The ether was evaporated and the solid residue was suspended in isopropanol. This mixture was heated on a steam bath for several minutes then allowed to cool to ambient temperature. The resulting powder was collected by filtration, washed with isopropanol, ether and dried (3.04 g 72.4%). NMR: 2.80 (s, 6H), 3.15 (m, 2H), 3.88 (m, 2H), 4.25 (m,1H), 5.93 (br S, 1H), 6.88 (m, 4H); m/z 211 $[MH]^+$; EA $C_{11}H_{18}N_2O_2$.1.6 HCl requires C; 49.2, H; 7.4, N; 10.4, Cl; 21.7%: found: C; 49.2, H; 7.2, N; 10.1; Cl; 19.1%.

Method 12

3-*N*,*N*-Dimethylamino-2-hydroxy-1-(4-nitrophenoxy)propane

1-(4-Nitrophenoxy)-2,3-epoxypropane (Method 13) (4.3 g) was dissolved in methanol (30 ml) and DMF (10 ml). Dimethylamine (2M solution in methanol, 17 ml) was added and the mixture was stirred at ambient temperature overnight. The reaction mixture was evaporated to dryness and the residue was dissolved in saturated sodium bicarbonate solution and ethyl acetate. The ethyl acetate layer was separated and washed twice with saturated brine, dried over anhydrous sodium sulphate, filtered and evaporated to yield an oil that slowly crystallised under high vacuum (4.79g, 89.9%). NMR ($CDCl_3$): 2.33 (s, 6H), 2.98 (m, 1H), 2.54 (m, 1H), 4.00 (m, 3 H), 7.00 (d, 2H), 8.20 (d, 2H); m/z 241 $[MH]^+$.

Method 13

1-(4-Nitrophenoxy)-2,3-epoxypropane

1-(4-Nitrophenoxy)-2,3-epoxypropane was prepared by an analogous method to that described by Zhen-Zhong Lui *et. al.* in Synthetic Communications (1994), *24*, 833-838.

4-Nitrophenol (4.0 g), anhydrous potassium carbonate (8.0 g) and tetrabutylammonium bromide (0.4 g) were mixed with epibromohydrin (10 ml). The reaction mixture was heated at 100°C for 1 hour. After cooling to ambient temperature, the reaction mixture was diluted with ethyl acetate and filtered. The filtrate was evaporated to dryness and the residue was co-distilled twice with toluene. The resulting oil was purified by column chromatography and eluted with ethanol (1.0%):dichloromethane to yield on evaporation an oil that crystallised (4.36 g, 77.7%). NMR ($CDCl_3$): 2.78 (m, 1H), 2.95 (m, 1H), 3.38 (m, 1H), 4.02 (dd, 1 H), 4.38 (dd, 1H), 7.00 (d, 2H), 8.20 (d, 2H); m/z 196 $[MH]^+$.

Method 14

2-Methylthio-4-(2,5-dimethylimidazo[1,2a]pyrid-3-yl)pyrimidine

A mixture of 3-(3-dimethylaminoprop-2-en-1-oyl)-2,5-dimethylimidazo[1,2a]pyridine (Method 15) (3.50g, 14.4mmol), thiourea (1.09g, 14.4mmol) and sodium methoxide (1.01g, 18.7mmol) were heated at 85°C in 1-butanol (50ml) for 2 hours. The mixture was allowed to cool to 30°C and methyl iodide (1.8ml, 28.8mmol) was added dropwise and the mixture stirred for a further 3 hours. The volatiles were removed by evaporation and the residue

urified by chromatography on silica eluting with ethyl acetate/methanol (100:0 increasing in polarity to 97:3) to give the title compound (2.37g, 61%). NMR: 2.41 (s, 3H), 2.60 (s, 3H), 2.70 (s, 3H), 7.56 (d, 1H), 7.88 (d, 1H), 7.92 (d, 1H), 8.81 (d, 1H), 9.39 (s, 1H); m/z: 271 $[MH]^+$.

Method 15

3-(3-Dimethylaminoprop-2-en-1-oyl)-2,5-dimethylimidazo[1,2a]pyridine

A solution of 3-acetyl-2,5-dimethylimidazo[1,2,a]pyridine (Method 16) (3.60g, 19.1 mmol) in dimethylformamide dimethyl acetal (20ml) was heated at reflux for 60 hours. The mixture was allowed to cool and the solvent was removed by evaporation. The residue was triturated with hot ether, the solid collected by filtration and dried to give the title compound (3.61g, 84%). NMR: 2.30 (s, 3H), 2.62 (s, 3H), 2.90 (br s, 3H), 3.10 (br s, 3H), 5.48 (d, 1H), 7.22 (dd, 1H), 7.44 (d, 1H), 7.68 (d, 1H), 9.39 (dd, 1H).

Method 16

3-Acetyl-2,5-dimethylimidazo[1,2a]pyridine

3-Chloro-2,4-pentanedione (6.5ml, 54.4mmol) was added to a suspension of 2-amino-4-methylpyridine (5.00g, 46.3mmol) and sodium iodide (10mg) in THF (60ml) and the mixture was heated at reflux for 16 hours. The reaction mixture was allowed to cool and the solvent was removed by evaporation. The resulting solid residue was triturated with hot hexane, collected by filtration and dried to give the title compound (3.69g, 43%). NMR: 2.35 (s, 3H), 2.75 (s, 3H), 7.41 (dd, 1H), 7.57 (d, 1H), 9.40 (d, 1H); m/z: 189 $[MH]^+$.

Method 17

4-(2-Methylpyrazolo[2,3a]pyrid-3-yl)-2-methylthiopyrimidine

A mixture of 3-(3-dimethylaminoprop-2-en-1-oyl)-2-methyl-pyrazolo[2,3a]pyridine (Method 18) (3.89g, 17mmol), thiourea (1.27g, 17mmol) and sodium methoxide (0.929g, 17mmol) in butanol (45ml) was heated at 85°C for two hours under nitrogen. Methyl iodide (1.05ml, 17mmol) was added and the mixture heated at 85°C for a further 2 hours. The volatiles were removed by evaporation and the residue was purified by chromatography on silica eluting with ethyl acetate/methanol (100:0 increasing in polarity to 97:3) to give the title

compound (3.1g, 68%). NMR: 2.58 (s, 1H), 2.68 (s, 3H), 7.04 (dd, 1H), 7.39 (dd, 1H), 7.
(d, 1H), 8.35 (d, 1H), 8.50 (d, 1H), 8.72 (d, 1H); m/z: 257 $[MH]^+$.

Method 18

3-(3-Dimethylaminoprop-2-en-1-oyl)-2-methylpyrazolo[2,3a]pyridine

A mixture of 3-acetyl-2-methyl-pyrazolo[2,3a]pyridine (Method 19) (2g, 11.5mmol) and *N,N*-dimethylformamide dimethyl acetal (10ml) was heated 110°C under nitrogen for 48 hours. The volatiles were removed by evaporation, the residue was triturated with hot ether and the solid product collected by filtration to give the title compound (1.98g, 75%). NMR: 2.60 (s, 3H), 3.30 (s, 6H), 5.49 (d, 1H), 6.95 (dd, 1H), 7.38 (dd, 1H), 7.62 (d, 1H), 8.10 (d, 1H), 8.62 (d, 1H); m/z: 230 $[MH]^+$.

Method 19

3-Acetyl-2-methylpyrazolo[2,3a]pyridine

Potassium carbonate (53.8g, 0.39mol) and then 2,4-pentanedione (24.8g, 0.25mol) were added to a solution of 1-aminopyridinium iodide (26.9g, 0.12mol) in water (336ml) and the mixture was heated at 80°C for 2 hours, allowed to cool to ambient temperature and left to stand for 18 hours. Water was added and the mixture was extracted to with ethyl acetate. The combined extracts were dried and the volatiles were removed by evaporation. The residue was recrystallized from hot hexane and the product collected by filtration. Solvent was removed from the filtrate by evaporation and was added to the insoluble residue from the recrystallization. This crude mixture was purified by chromatography on silica eluting with dichloromethane/hexane (1:1) increasing in polarity to dichloromethane/methanol (97:3). This product was triturated with hexane and added to the product obtained from the initial recystallisation to give the title compound (9.6g, 33%). NMR: 2.50 (s, 3H), 2.62 (s, 3H), 7.09 (dd, 1H), 7.55 (dd, 1H), 8.12 (d, 1H), 8.72 (d, 1H); m/z: 175 $[MH]^+$.

Example 39

The following illustrate representative pharmaceutical dosage forms containing the compound of formula **(I)**, or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof (hereafter compound X), for therapeutic or prophylactic use in humans:-

a): Tablet I	mg/tablet
Compound X	100
Lactose Ph.Eur	182.75
Croscarmellose sodium	12.0
Maize starch paste (5% w/v paste)	2.25
Magnesium stearate	3.0

(b): Tablet II	mg/tablet
Compound X	50
Lactose Ph.Eur	223.75
Croscarmellose sodium	6.0
Maize starch	15.0
Polyvinylpyrrolidone (5% w/v paste)	2.25
Magnesium stearate	3.0

(c): Tablet III	mg/tablet
Compound X	1.0
Lactose Ph.Eur	93.25
Croscarmellose sodium	4.0
Maize starch paste (5% w/v paste)	0.75
Magnesium stearate	1.0

(d): Capsule	mg/capsule
Compound X	10
Lactose Ph.Eur	488.5
Magnesium stearate	1.5

(e): Injection I	(50 mg/ml)
Compound X	5.0% w/v
1M Sodium hydroxide solution	15.0% v/v
0.1M Hydrochloric acid	(to adjust pH to 7.6)
Polyethylene glycol 400	4.5% w/v
Water for injection	to 100%

(f): Injection II	10 mg/ml
Compound X	1.0% w/v
Sodium phosphate BP	3.6% w/v
0.1M Sodium hydroxide solution	15.0% v/v
Water for injection	to 100%

(g): Injection III	(1mg/ml,buffered to pH6)
Compound X	0.1% w/v
Sodium phosphate BP	2.26% w/v
Citric acid	0.38% w/v
Polyethylene glycol 400	3.5% w/v
Water for injection	to 100%

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

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